Developmental Cell *13*

Supplemental Data

Regulated HsSAS-6 Levels Ensure Formation of a Single Procentriole per Centriole during the Centrosome Duplication Cycle

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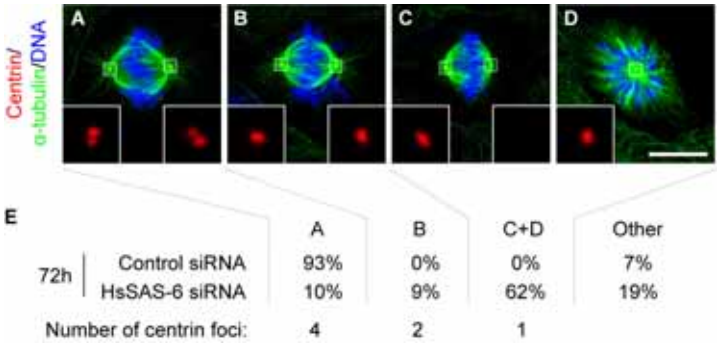

Figure S1. HsSAS-6 Is Required for Procentriole Formation in U2OS Cells

(A-D) U2OS cells transfected with control siRNAs or siRNAs directed against HsSAS-6, fixed 72 hours after transfection and stained with antibodies against centrin (red) and α-tubulin (green); DNA is shown in blue. Insets are magnified 5-fold. Scale bar: 10 μm. Cells were classified into the following categories: two pairs of centrin foci, bipolar spindle (A); two single centrin foci, bipolar spindle (B); one single centrin focus, bipolar (C) or monopolar (D) spindle. (E) Distribution of cells in categories described above (n=100); Other: cells that could not be assigned to any of the four categories (~ 7% of cells that contained more than 4 centrin foci, for both experimental conditions, as well as some cells containing a pair of juxtaposed centrin foci in the case of cells targeted with HsSAS-6 siRNAs).

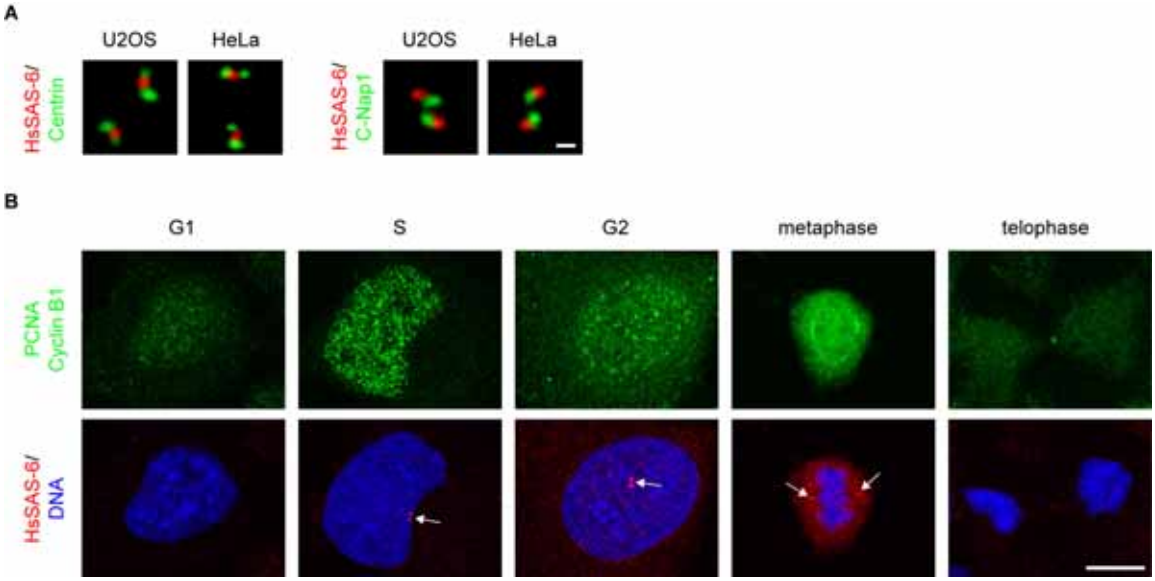

Figure S2. HsSAS-6 Cell Cycle Regulation and Localization in U2OS and HeLa Cells

(A) U2OS and HeLa cells were stained with antibodies against HsSAS-6 (red) and centrin (green) or C-Nap1 (green). Note that the position of HsSAS-6 foci relative to centrin and C-Nap1 is identical in both cell lines. Scale bar: 500 nm.

(B) U2OS cells stained with antibodies against PCNA, Cyclin B1 (both in green; top) and HsSAS-6 (red; bottom); DNA is shown in blue (bottom). Scale bar: 10μm. Arrows point to centriolar HsSAS-6.

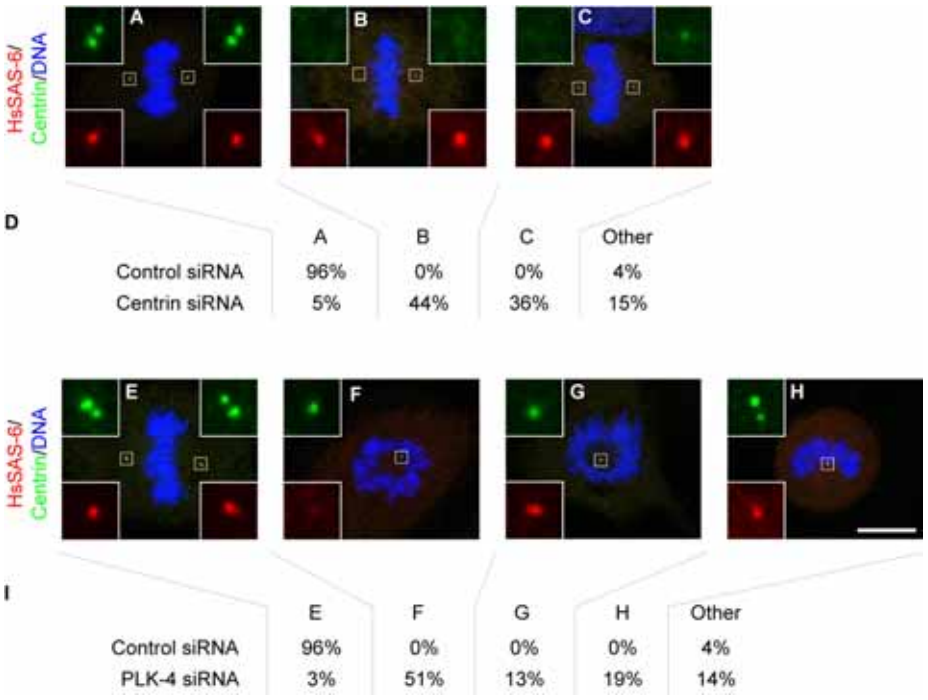

Figure S3. HsSAS-6 is Recruited to Centrioles Independently of Centrin-2 But Plk4 Contributes to HsSAS-6 Centriolar Maintenance

(A-D) HeLa cells transfected with control siRNAs or siRNAs directed against centrin-2, stained with antibodies against centrin (green) and HsSAS-6 (red); DNA is shown in blue. Insets are magnified 5-fold. Note that centrin-2 was undetectable by immunofluorescence analysis following siRNA-medidated depletion. Nevertheless as reported previously (Salisbury et al., 2002) such cells invariably formed bipolar spindles. Together with our observation that centriolar HsSAS-6 is present in such cells, this raises the possibility that procentriole formation can proceed at the least to some extent following centrin-2 depletion.

(E-I) HeLa cells transfected with control siRNAs or siRNAs directed against Plk4, stained with antibodies against centrin (green) and HsSAS-6 (red); DNA is shown in blue. Insets are magnified 5-fold. Scale bar: 10 μm. 100 cells were scored in D and I.

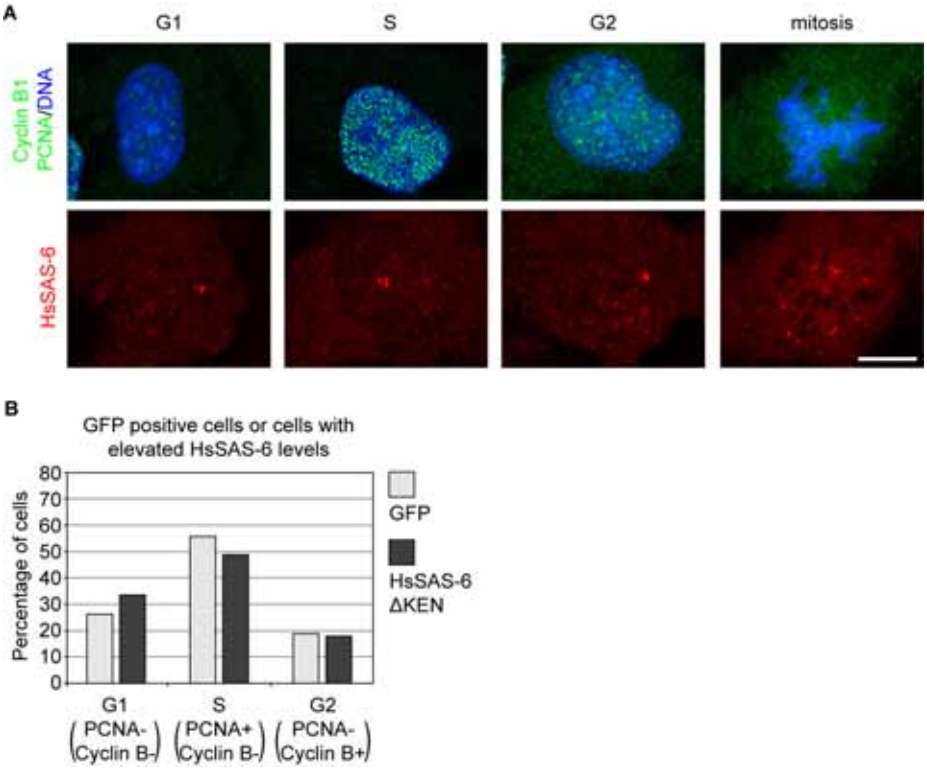

Figure S4. HsSAS-6-ΔKEN Is Expressed in G1 Cells and Does Not Affect Cell Cycle Progression

(A) U2OS cells transfected with GFP or HsSAS-6-ΔKEN, fixed after 72h and stained with antibodies against PCNA, Cyclin B1 (both green; top) and HsSAS-6 (red; bottom) or GFP (not shown); DNA is shown in blue (top). Note the mitotic cell with multiple foci of HsSAS-6. Scale bar: 10 μm.

(B) Cells were treated and processed as in (A) and the PCNA and Cyclin B1 staining evaluated in GFP positive cells (for cells transfected with GFP) or in cells with elevated HsSAS-6 level (for cells transfected with HsSAS-6-ΔKEN) (n=100). Note that G1 cells expressing elevated levels of HsSAS-6-ΔKEN were present, underscoring the fact that the protein is not efficiently degraded during this phase of the cell cycle.

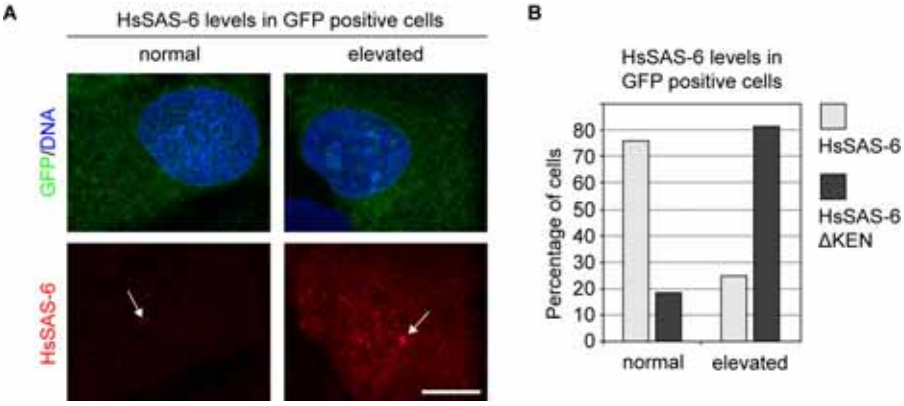

Figure S5. HsSAS-6-ΔKEN Accumulates More Than HsSAS-6

(A) U2OS cells cotransfected with GFP and HsSAS-6 (left) or HsSAS-6-ΔKEN (right), fixed after 72h and stained with antibodies against GFP (green) and HsSAS-6 (red); DNA is shown in blue. Representative images of cells with normal (left) or elevated (right) HsSAS-6 levels are shown. Scale bar: 10 μm.

(B) Cells were treated and processed as in (A) and the level of HsSAS-6 in GFP positive cells was scored (n=100). Note that while protein levels are normal in most cells transfected with HsSAS-6, they are elevated in most cells transfected with HsSAS-6-ΔKEN, presumably reflecting lack of efficient degradation. In the case of HsSAS-6 overexpression, cells that were categorized as normal included some G1 cells in which the protein was not detectable, as is the case for the endogenous protein.

Movies S1–S3. HsSAS-6 Degradation Is Dependent on a KEN Box

Dual GFP fluorescence and phase contrast time lapse microscopy of HeLa cells expressing GFP-HsSAS-$6^{N\text{-}ter}$ (M1), GFP-HsSAS-$6^{C\text{-}ter}$ (M2) and GFP-HsSAS-$6^{C\text{-}ter\text{-}\Delta KEN}$ (M3). Time is shown in min; 0 corresponds to the metaphase to anaphase transition.

Supplemental Reference

Salisbury, J. L., Suino, K. M., Busby, R., and Springett, M. (2002). Centrin-2 is required for centriole duplication in mammalian cells. Curr Biol *12*, 1287-1292.